Enhanced IL-4 Production and IL-4 Receptor Expression in Atopic Dermatitis and Their Modulation by Interferon-Gamma

Harald Renz, Kazuhito Jujo, Katherine L. Bradley, Joanne Domenico, Erwin W. Gelfand, and Donald Y.M. Leung

Department of Pediatrics, Divisions of Basic Sciences and Allergy-Immunology and the Raymond and Beverly Sackler Foundation, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, U.S.A.

The in vivo and in vitro immunomodulatory effects of interferon gamma (IFN-γ) treatment on peripheral blood mononuclear cells (PBMC) from patients with atopic dermatitis (AD) and elevated IgE levels were studied. As part of a double-blind placebo-controlled clinical trial, 14 AD patients were treated with IFN-γ ($n = 7$) or saline ($n = 7$) for 12 weeks. To assess the in vivo effects of IFN-γ treatment on interleukin (IL)-4–dependent lymphocyte function, we assessed the proliferation of AD PBMC in response to IL-4. Prior to IFN-γ treatment, AD PBMC had proportionately decreased proliferative responses to IL-4 when compared to IL-2. After 12 weeks of in vivo treatment with IFN-γ, there was an increase of IL-4– but not IL-2–induced lymphocyte proliferation in seven of eight AD patients.

To further study the immunologic basis of these observations, we studied the expression of IL-4 receptor (IL-4R) mRNA and the production of IL-4 by PBMC from AD patients. PBMC from AD patients expressed higher levels of IL-4R mRNA and produced significantly higher amounts of IL-4 than normal controls ($p < 0.05$). More importantly, the in vitro addition of IFN-γ caused significant reduction in both IL-4R mRNA expression and IL-4 production of PBMC from AD and non-atopic controls.

These data indicate that AD is characterized by an in vivo overstimulation of the IL-4–IL-4R pathway. The poor proliferative responses of untreated AD PBMC to exogenous IL-4 may be due to increased levels of endogenous IL-4 production with constant occupancy of the IL-4R. Furthermore, in vivo and in vitro treatment with IFN-γ down-regulates this pathway. *J Invest Dermatol 99:403–408, 1992*

Atopic dermatitis (AD) is a chronic inflammatory skin disease that is frequently associated with markedly elevated serum immunoglobulin (Ig)E levels [1–3]. T lymphocytes are thought to play an important role in the pathogenesis of this disease [4]. It is now well established that interleukin (IL)-4 acts as an isotype switch factor for the induction of IgE synthesis [5–7]. In contrast, IFN-γ inhibits IL-4–induced IgE production [8–12]. Studies of the murine immune system have demonstrated that these cytokines are produced by different T-helper (TH) cell subpopulations [13]. It has also been found that IL-2 and IL-4 are intimately involved in the regulation of lymphocyte proliferation. As shown in several studies in human and mice, IL-2 and IL-4 are able to induce lymphocyte growth in an equal fashion [14–16] and, as demonstrated recently by our group and others, both IL act through independent pathways [17–20].

Recently, there have been several reports that PBMC from patients with AD produce decreased levels of IFN-γ paralleled by an increase in IL-4 production [21–23]. Furthermore, a high frequency of IL-4 producing but not IFN-γ–producing CD4+ allergen-specific T lymphocytes infiltrate into the inflammatory skin lesion of AD [24]. Such studies have provided the rationale for clinical trials of recombinant (r) IFN-γ in the treatment of AD [25]. In a recent multi-center double-blind placebo-controlled trial, AD patients treated with rIFN-γ had a significantly greater reduction in clinical severity than patients treated with placebo [26]. As part of this clnical trial, we assessed the in vivo and in vitro effects of IFN-γ treatment on the modulation of IL-4–dependent lymphocyte functions. Our current study demonstrates that AD is characterized by an in vivo upregulation of the IL-4–IL-4R pathway, which, in turn, is under the negative regulatory control of IFN-γ.

MATERIALS AND METHODS

Study Population Fourteen patients with severe AD, who were enrolled as part of a double-blind placebo-controlled study assessing the efficacy of rIFN-γ in AD, were studied [26]. All patients or guardians gave written informed consent prior to participation in the study. Patients were between the ages of 7 and 53 years and had severe AD diagnosed according to the criteria of Hanifin et al [27]. Ten non-atopic healthy age-matched volunteers were studied as controls.

In Vivo rIFN-γ Treatment AD patients enrolled in the double-blind placebo-controlled clinical trial were assigned to rIFN-γ or placebo treatment by a randomized sequence generated by the Gen-

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Reprint requests to: Dr. Donald Y.M. Leung, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St, Denver, CO 80206.

Abbreviations:
AD: atopic dermatitis
ELISA: enzyme-linked immunosorbent assay
IFN-γ: interferon gamma
Ig: immunoglobulin
IL-2/IL-2R: interleukin 2/interleukin 2 receptor
IL-4/IL-4R: interleukin 4/interleukin 4 receptor
MCF: mean channel fluorescence
PBMC: peripheral blood mononuclear cells
PBS: phosphate-buffered saline
PHA: phytohemagglutinin
r: recombinant

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entech, Inc., Biostatistics group [26]. rIFN-γ was received in a single daily subcutaneous injection of 50 μg/m^2 for 12 weeks. rIFN-γ was supplied as a sterile solution ready for parenteral administration in 0.1 mg/vial at a concentration of 0.2 mg/ml. Placebo was received by the same regimen and was supplied in an identical configuration and contained an equal volume of IFN-γ diluent. Patients were permitted to use low- to medium-potency topical corticosteroids during the study. No patient was on systemic corticosteroids for at least 1 month prior to the study entry. All patients completed the study. Decoding at the end of the study revealed that seven AD patients had received rIFN-γ and seven had received placebo. The mean age and the severity of the disease were comparable in both groups. All immunologic assays were performed in a blinded fashion without knowledge about the treatment of the individual patients.

Blood Sampling and Cell Preparations Heparinized blood was drawn at the beginning of the study and after receiving the last injection at the end of the 12-week trial. Peripheral blood mononuclear cells (PBMC) were prepared by Ficoll-Hypaque gradient centrifugation for 15 min at room temperature. The interphase was collected and washed three times in PBS for 5 minutes at 1500 rpm. Cells were counted and resuspended in RPMI 1640 tissue culture medium (Gibco, Grand Island, NY), supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (5 mM), L-glutamine (2 mM), and 10% heat-inactivated fetal calf serum.

Lymphocyte Proliferation Assay The proliferative responses to IL-2 and IL-4 were assayed by incubating 0.2×10^6 PBMC in complete tissue culture medium in 96-well flat-bottom culture plates (Falcon, Lincoln Park, NY) for 72 h. Cells were pre-activated by addition of PHA in a concentration of 500 ng/ml, which was found to be a submitogenic dose [17]. IL-2 (kindly provided by Cetus, Emeryville, CA) was added in a concentration of 10 U/ml and IL-4 (kindly provided by Dr. Paul Trotta, Schering-Plough Research Institute, Bloomfield, NJ) was added at 200 U/ml. Both concentrations were found to induce maximal proliferation in normal donors. For at least 4–6 h [^{3}H]thymidine (Amersham Corp., Arlington Heights, IL) was added (1 μCi/well). Cells were harvested on a multi-channel Cambridge PhD cell harvester. [^{3}H]TdR incorporation was measured in a beta scintillation counter.

Analysis of IL-4R mRNA by Northern Blotting Total cellular RNA was isolated by lysing cells in 300 mM NaAc, pH 5.5, 10 mM EGTA, pH 8, 0.2% SDS, and 10 mg Na heparin Grade I (Sigma) as an RNAse inhibitor. Cell lysates were extracted once with phenol-chloroform at 65°C followed by a second extraction at room temperature. Nucleic acids were ethanol precipitated and high-molecular-weight material was removed by centrifugation at 1000 rpm for 1 min at room temperature. For Northern blotting, RNA (7.5 μg/lane) was separated by electrophoresis in 1% agarose gels containing formaldehyde and blotted onto nylon membranes (Micron Separations, Inc., Westboro, MA). Membranes were prehybridized for at least 5 h at 42°C in 50% formamide, 5 × SSC, 0.05 M NaPhosphate, 0.5% SDS, and 50 μg/ml salmon sperm DNA. Hybridization was performed overnight under the same conditions with a human IL-4R probe, as described [28,29], using the RNA transcription test (Stratagene, La Jolla, CA). The IL-4R probe was kindly provided by Dr. M. Patricia Beckmann, Immunex Corp., Seattle, WA. The GAPDH probe was provided by Dr. Nishida, Kobe, Japan, and equivalent amounts of RNA/gel were assessed by monitoring dehydrogenase GAPDH mRNA levels.

Determination of IL-4 Production in Culture Supernatants IL-4 could not be reliably measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits. We therefore assessed IL-4 content by the capacity of supernatants to induce CD23 on the CD23-negative Ramos B-cell line based on a biologic assay described by Custer and Lotze [30]. Ramos cells were subcultured with RPMI 1640 in complete culture medium. For experiments, only viable cells obtained from Ficoll-hypaque gradient centrifugation were used. Cells were cultured in 96-well tissue culture plates (Falcon) in a concentration as 1×10^5 cells per well and stimulated with 5 μg/ml of anti-IgM (Tago, Burlingame, CA). Either human rIL-4 in a concentration range between 1 and 100 U/ml or dilutions of tissue culture supernatants were added for 48 h. Cells were washed twice and reincubated with human immunoglobulins at 10 mg/ml in order to prevent non-specific binding by Fc receptor. Subsequently, the cells were incubated with monoclonal anti-CD23 antibody for 30 min in staining buffer [phosphate-buffered saline (PBS) supplemented with 2% heat-inactivated fetal calf serum and 0.1% sodium azide]. After washing three times, CD23 expression was determined for each sample and standard. A standard curve was then established for each assay by plotting of CD23 mean channel fluorescence (MCF) value against corresponding IL-4 concentrations of standard samples on a linear scale. Using this standard curve, MCF values of samples analyzed was transformed into IL-4 concentrations. The specificity of this assay was demonstrated by the loss of CD23 induction on Ramos cells when samples were preincubated with anti-IL-4 antibodies. IFN-γ did not interfere with the IL-4 assay because MoAb GIR208 (gift from Dr. R. Schreiber, St. Louis), which blocks the binding of IFN-γ to its receptor, had no effect on the induction of CD23 by culture supernatants. The sensitivity range of this assay ranged from 0.5 U/ml up to 100 U/ml of 72-4; 16 supernatants contained higher amounts of IL-4. Further dilutions of the supernatants were analyzed.

The following experiments were carried out to demonstrate the specificity of the assay: samples tested in the presence of polyclonal anti-IL4 (Genzyme, MA) lost their CD23-inducing activity. Because IFN-γ is able to downregulate CD23 expression, all samples were preincubated with anti-IFN-γ antibody (Genentech, CA) to neutralize any IFN-γ activity in the sample. Furthermore, addition of purified IgE (up to 50 ng/ml) to Ramos cells whose CD23 expression was induced by IL-4 did not affect the MCF values for CD23.

Statistical Analysis Data were analyzed by two-tailed Student t test for unpaired samples and by the chi-squared test (Fig 1).

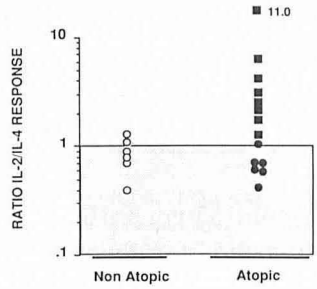

Figure 1. Ratios of IL-2:IL-4 dependent lymphocyte proliferation in 14 AD patients and six age-matched controls. PBMC (2×10^5) per well were preactivated with a submitogenic concentration of PHA (500 ng/ml) and further stimulated with either IL-2 (10 U/ml) or IL-4 (200 U/ml) for 3 d. The ratio of [^{3}H] thymidine incorporation of IL-2- and IL-4-treated cultures was determined for each individual. The values between the groups are significantly ($p < 0.05$) different.

RESULTS

Impaired Proliferative Response to IL-4 in AD The proliferative responses to exogenous IL-2 and IL-4 was examined on PBMC from 14 atopic patients and six age-matched normal controls. The induction of lymphocyte responsiveness to interleukins requires cell preactivation. This was achieved by stimulation with a submitogenic concentration of phytohemagglutinin (PHA) 500 ng/ml). As shown previously [17], in this way preactivated lymphocytes proliferate in an equal fashion to optimal concentrations of IL-2 (10 U/ml) and IL-4 (200 U/ml). This results in an IL-2/IL-4 ratio of about 1. Table I shows the proliferative response of one representative

Table I. Proliferative Responses of PBMC from One Normal Donor and Atopic Dermatitis Patient[a]

	Normal	Atopic Dermatis
Control	1065 ± 600	1470 ± 405
PHA (500 mg/ml)	5257 ± 1800	4446 ± 1360
PHA + IL-2 (10 U/ml)	52,010 ± 3980	67,486 ± 5840
PHA + IL-4 (200 U/ml)	54,030 ± 3860	10,311 ± 690

[a] Cells were cultured with medicated conditions as described in *Materials and Methods* for 72 h [^{3}H] thymidine was added for the last 6 h. The data are expressed as mean ± SD from triplicate cultures.

normal and atopic donor to the stimulation of PHA-preactivated PBMC with IL-2 and IL-4. The incubation with the submitogenic concentration of PHA resulted in [^{3}H]TdR incorporation of less than 5% of the maximal counts observed in the individual donors.

In non-atopic subjects, the responses to IL-2 and IL-4 did not differ significantly from each other. Atopic subjects always showed a response to the addition of IL-2 comparable to the one measured in the control group. But, in contrast to non-atopic volunteers, 9 of the 14 AD patients exhibit an IL-2/IL-4 ratio that was greater than 1 ($p < 0.05$ compared to normal). IL-2/IL-4 ratios greater than 1 were always the result of a decreased proliferative response to IL-4 and a normal proliferative response to IL-2 (Fig 1). The remaining patients were characterized by a similar response to both interleukins compared with the non-atopic donors, as indicated by a ratio of about 1.

In Vivo rIFN-γ Treatment Normalizes IL-4 – Dependent Proliferation of AD PBMC As part of a multi-center double-blind placebo-controlled trial, we reported that treatment with rIFN-γ results in significantly greater clinical improvement of AD signs and symptoms than placebo treatment [26]. PBMC from 14 patients who were part of this clinical trial were examined for their in vitro proliferative response to both IL-2 and IL-4 before the trial was started, and after 12 weeks of IFN-γ or placebo treatment. Figure 2 displays the IL-2/IL-4 ratios for each individual patient in the IFN-γ and placebo groups before and after treatment. Treatment with IFN-γ significantly reduced the IL-2/IL-4 ratio of AD patients ($p = 0.02$). In contrast, no significant changes were observed in the placebo-treated group ($p = 0.8$). The decrease of IL-2/IL-4 ratio in the treated group was due to an increase of IL-4 – dependent lymphocyte proliferation, which increased between 200% and 500% (Fig 3). In contrast, the IL-2 – induced response showed no significant changes. These data indicate that in vivo IFN-γ treatment increases the responsiveness of atopic PBMC to IL-4.

IL-4 Production and IL-4 Receptor (IL-4R) are Enhanced in AD In order to examine whether the decreased in vitro proliferative response to IL-4 may be related to an altered regulation of IL-4 production or IL-4R expression in AD, the spontaneous and PHA-induced release of IL-4 from PBMC was examined. For this purpose, PBMC from AD patients and controls were incubated with or without PHA (10 μg/ml). The kinetics of IL-4 release from PHA-activated PBMC revealed that IL-4 production increased during the first 24 h, followed by a plateau during the second 24-h period (data not shown). Based on these kinetic experiments, the production of IL-4 was measured in 24-h culture supernatants.

In unstimulated cultures from normal control donors, only two of nine donors had any detectable IL-4 production. In contrast, in six of nine AD patients spontaneous IL-4 production of up to 22 U/ml was detected (data not shown). Table II shows the results on IL-4 production in PHA-stimulated cultures from two representative normal control donors and two patients with AD. Although PHA induced IL-4 production in all cases, significantly higher amounts were detected in supernatants from patients with AD ($p < 0.05$). These data indicate that AD is associated with increased spontaneous and PHA-stimulated IL-4 production.

As it has been demonstrated that IL-4 is an important stimulator

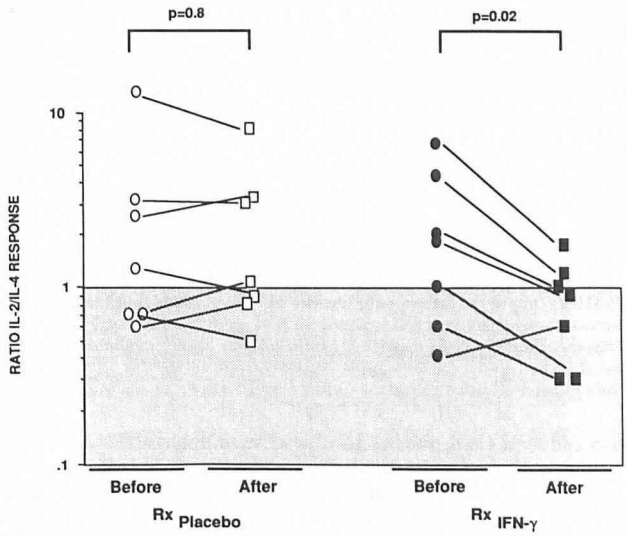

Figure 2. Effects of IFN-γ or placebo treatment on IL2/IL4 proliferative response in AD patients. The data were expressed as the ratio for each individual before and after completion of the clinical trial. Patients were assigned to IFN-γ or placebo treatment as described in *Materials and Methods*. The individual proliferative responses to IL-2 and IL-4 were determined as described in Fig 1.

for the up-regulation of its own receptor [32], the expression of IL-4R was studied on the mRNA level by Northern blot analysis. The analysis of IL-4R mRNA indicated that freshly isolated PBMC from AD patients expressed higher amounts of IL-4R mRNA than the controls (Fig 4*A*). Stimulation with PHA for 48 h enhanced IL-4R mRNA expression in both atopic and control subjects (Fig 4*B*).

In Vitro Treatment of PBMC with IFN-γ Reduces IL-4 Production and IL-4R Expression It has been shown that IFN-γ antagonizes a number of IL-4 – dependent lymphocyte functions including the induction of IgE synthesis [18 – 22]. The actual mechanism by which IFN-γ acts, however, is unknown. To examine whether the production of IL-4 and the up-regulation of IL-4R may be sensitive to rIFN-γ, PHA-stimulated PBMC from AD patients and normal subjects were incubated in the presence and absence of rIFN-γ. Table II shows the effect of IFN-γ on PHA-induced IL-4 production in PMBC from atopic and normal subjects. In all cases, IFN-γ inhibited PHA-induced IL-4 production. This effect was observed in all of the nine studied AD patients as well as in four of four PBMC cultures of controls. In both groups, the IL-4 down-regulation by IFN-γ was signficant ($p < 0.05$). To some degree, IFN-

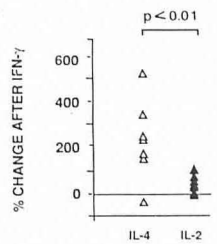

Figure 3. Treatment with rIFN-γ increases the proliferative response to IL-4 but not to IL-2. For the seven IFN-γ – treated patients, the differences in the proliferation patterns to IL-4 and IL-2, before and after treatment, are expressed as percent changes. The response at the beginning of the trial was calculated as 100%. IL-4 – and IL-2 – induced lymphocyte proliferation was performed as described in Fig 1. The changes in IL-4 – induced proliferation are significantly higher ($p < 0.01$) than for IL-2.

Table II. IFN-γ Inhibits Spontaneous and PHA-Induced IL-4 Production[a]

	IL-4 (U/ml)	
	−IFN-γ	+IFN-γ
N1	126 ± 18	17 ± 6
N2	175 ± 36	25 ± 12
AD1	578 ± 61	184 ± 21
AD2	638 ± 112	102 ± 31

[a] PBMC from 2 atopics (AD) and 2 normal controls were cultured with medium and PHA (10 μg/ml) in the presence and absence of IFN-γ (200 U/ml) for 24 h. Culture supernatants were harvested and analyzed for IL-4 production as described in *Materials and Methods*. Shown are the mean ± SD for triplicates of each condition.

γ also reduced IL-4 production by unstimulated PBMC from AD patients, but no significant differences were found. Similarly, the PHA-induced up-regulation of the IL-4R was also found to be sensitive to IFN-γ (Fig 4*B*). PHA stimulation induced IL-4R expression to a similar extent in atopic and normal PBMC. In both groups reduction of IL-4R expression was observed when the cells were co-treated with IFN-γ.

DISCUSSION

This study demonstrates that IL-4R expression and IL-4 production are under the regulatory control of IFN-γ. PBMC of AD patients exhibit higher IL-4 production and increased IL-4R mRNA expression than normal subjects. AD represents a disease that is characterized by enhanced IgE production [1–3]. Both IL, IL-4, and IFN-γ have been shown to be intimately involved in the regulation of IgE production. IL-4 acts as a switching factor for IgE [5–7] and IFN-γ counteracts the IgE stimulatory effects of IL-4 [8–12]. It has been demonstrated that IFN-γ inhibits IgE production by directly influencing B-cell functions [8], as well as by influencing T-cell functions [7]. Taken together, these observations suggest that AD represents a disease in which an imbalance between the production and functions of IL-4 and IFN-γ may be important. To further explore these interactions, we examined possible effects of IFN-γ in vivo and in vitro on IL-4 production and IL-4R expression.

IL-4 acts through interaction with its own receptor, the IL-4R. Resting lymphocytes constitutively express IL-4R [29]. We studied the expression of IL-4R on the mRNA level by Northern blot analysis. Freshly prepared unstimulated PBMC from patients with AD expressed higher amounts of IL-4R mRNA. It has been previously demonstrated that IL-4 acts as a potent upregulator of its own receptor [28,31,32]. Because IL-4 production in AD is enhanced compared to normal control subjects [22,23], it is possible that the increased IL-4 production accounts for this enhanced in vivo expression of IL-4R. This dysregulation ultimately may result in the hyper-production of IgE observed in these patients.

Analysis of the proliferative response to IL-4 surprisingly revealed a diminished proliferative response to this lymphokine in more than half the cases of AD. One possible explanation for this diminished proliferative response to IL-4 may be that, constitutively, in vivo production of IL-4 leads to constant occupancy of the IL-4R and, in turn, when lymphocytes were re-exposed in vitro to IL-4, the cells could not respond with a normal proliferation pattern. This possibility is supported by the observation that other IL-4–dependent lymphocyte functions are also impaired in AD. In this regard, Vollenweider et al [33] reported that although in normal PBMC IL-4 stimulated IgE production, none or only a marginal enhancement of IgE synthesis by IL-4 was reported in PBMC from AD patients. These data, together with our observations, may indicate that IL-4–dependent pathways are already stimulated in vivo and that further in vitro exposure to IL-4 exhibits only small effects.

The data presented indicate that the production of IL-4 and IL-4R are under the negative regulatory control of IFN-γ. In vitro we found that IFN-γ reduced enhanced IL-4R expression at the mRNA level. As the available data from humans and mice indicate that IL-4R mRNA production is strongly paralleled by IL-4R protein production and cell-surface expression [28,30,32], it may be concluded that the down-regulation of IL-4R mRNA leads to a reduction in the surface expression of IL-4R as well. Whether the reduction at the mRNA level is due to increased degradation or reduced transcription of IL-4 mRNA requires further study. IL-4 was measured in culture supernatants employing a bioassay that is based on the induction of CD23 expression by IL-4 on Ramos cells. This assay was originally described by Custer and Lotze [30] and combines a number of advantages. The induction of CD23 on this cell line is specifically induced only by IL-4, as other tested interleukins, including IL-6 and GM-CSF (which also effect CD23 expression), failed to do so on Ramos cells. Moreover, a possible inhibitory effect of IFN-γ on CD23 was extended by the addition of an anti–IFN-γR antibody to selected samples. As presentation of IL-4–containing samples with anti–IL-4 prevented the induction of CD23 expression, this further underlines the specificity of this assay.

We also report that treatment of AD patients for 12 weeks with rIFN-γ resulted in an increase of IL-4–dependent lymphocyte proliferation in all but one individual. This reversal from pre-treatment findings may be related to a decrease in IL-4 production during the treatment period. Because the IL-4–IL-4R axis was found to be overstimulated in untreated patients, the extrapolation of our in vitro data may predict a similar inhibitory activity of IFN-γ on IL-4 production and IL-4R expression in vivo. This would result in a normalized response to exogenously added IL-4 in the proliferation assay.

One explanation for the overstimulation of the IL-4–IL-4R pathway in AD may be a dysfunction in IFN-γ production that results in the lack of an important inhibitory factor for IL-4–IL-4R expression. In fact, we and others [21–23] demonstrated that AD patients show diminished IFN-γ production upon in vitro stimulation of PBMC. It has been reported that there is also a second negative feedback loop between IL-4 and IFN-γ, i.e., IL-4 inhibits IFN-γ production at least under certain culture conditions [34,35].

A second explanation for enhanced IL-4 production and diminished IFN-γ secretion by PBMC from allergic patients may be that these diseases are characterized by an imbalance in IL-4 and IFN-γ–producing T cells in peripheral blood. Indeed, studies on T-cell clones from allergic patients indicated an increased frequency of IL-4–secreting and a decreased number of IFN-γ–producing allergen-specific T-cell clones [37,38]. Although this shift in the frequency of IL-4– and IFN-γ–producing T-cell clones was reported by several groups, it still remains unclear whether freshly isolated T cells from allergic patients exhibit this biased pattern of cytokine production.

Taken together, our observations suggest the following sequence of events. First, the cytokine profile in AD is characterized by endog-

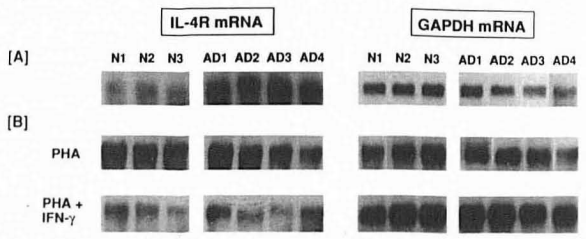

Figure 4. Analysis of IL-4R mRNA expression in three controls (N1–N3) and 4 AD patients (AD1–AD4). IL4R mRNA was analyzed on fresh PBMC *(A)* and after 48-h stimulation with PHA (10 μg/ml) in the presence or absence of IFN-γ (200U/ml) *(B)*. Equal loading of the gels was demonstrated by hybridization with the GAPDH housekeeping gene.

enous overproduction of IL-4. Second, this hyperproduction of IL-4 leads to the increased expression of IL-4R. Third, this sequence is enhanced due to the reduced IFN-γ production in AD, either as a result of lower frequencies of IFN-γ-producing T cells or secondary to the inhibitory activity of IL-4 on IFN-γ production. In either case, these studies indicate that treatment of PBMC with IFN-γ reduces IL-4 production and IL-4R expression in AD, thereby restoring responsiveness of AD PBMC to IL-4. These data provide some explanation for the clinical efficacy of IFN-γ treatment in AD and provide a rationale for further investigations into the role of IFN-γ in allergic diseases.

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ANNOUNCEMENT

The International Summit on Cutaneous Antifungal Therapy will be held October 21–24, 1993 at the The Ritz-Carlton San Francisco Hotel.

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For further information, please contact Skin Diseases Research Center, 2074 Abington Road, Cleveland, Ohio 44106-5000; Tel. 216-844-3682; Fax 216-844-8993.